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Skin type VII collagen acts as a tumour suppressor by regulating TGFβ and angiogenesis

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Why individuals with severe generalized recessive dystrophic epidermolysis bullosa (RDEB), a rare inherited blistering disorder caused by mutations in the COL7A1 gene, develop aggressive squamous cell carcinomas (SCC) is unexplained. Here we report that loss of type VII collagen (Col7) in SCC results in increased angiogenesis in vitro and in vivo with increased TGFβ signalling. Stable knockdown (KD) of Col7 was established using shRNA and cells were used in a mouse xenograft model (n=4 to 7 per group). Angiogenesis was assessed by measuring vessels in tumour sections, using endothelial cell tube-forming assays and by proteome arrays. Increased angiogenesis and VEGF expression was observed in Col7 KD xenografts and in RDEB tumors (n=21) compared to sporadic SCC tumours (n=24). Recombinant human Col7 reversed the increased SCC angiogenesis in mouse and zebrafish xenografts. Increased TGFβ-responsive promoter activity was observed in Col7 KD SCC cells. Blocking the interaction between α2β1 integrin and Col7, but not α2β1 integrin and Col4, with an α2-neutralising antibody increased *TGFB1* mRNA and increased kindlin2 and αVβ6 expression, known drivers of TGFβ signalling. Increased nuclear Smad4 was expressed at the edge of invading shCol7 xenografts, with loss of the cytoplasmic staining observed in the presence of Col7. Inhibition of TGFβ receptor signaling, using siRNA or a specific inhibitor, resulted in decreased endothelial cell tube formation and decreased VEGF secretion Our findings demonstrate that type VII collagen suppresses TGFβ signaling and angiogenesis in cutaneous SCC. Patients with RDEB SCC, who have little or no type VII collagen, may benefit from anti-angiogenic therapy.

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Nuclear actin controls keratinocyte motility via transcriptional regulation of adhesive and cytoskeletal genes

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The actin cytoskeleton is a classic regulator of cellular mechanics and mechano-sensing, and dynamically responds to biophysical cues through rapid polymerisation, de-polymerisation, and the generation of acto-myosin contractile forces. While it is well established that actin also shuttles in and out of the nucleus, the regulatory mechanisms controlling these processes and the function of actin in the nucleus remain unclear. In this study, we investigated how adhesive cues from the extra-cellular matrix regulate nuclear actin dynamics and the impact of nuclear actin on keratinocyte gene expression and cell behaviour. Analysis of nuclear actin turnover using fluorescence recovery after photo-bleaching (FRAP) revealed that limited cell adhesion on micro-patterned surfaces did not alter the rate of actin transport through the nucleus but increased the steady state level of actin within the nucleus, and this response depended on overall actin polymerisation within the cytoplasm. We next performed gene expression profiling in cells overexpressing beta-actin tagged with a nuclear localisation sequence (NLS-actin), which forced actin to accumulate within the nucleus. Compared to parental cells and cells overexpressing wild type actin, NLS-actin keratinocytes displayed reduced expression of multiple adhesion and cytoskeletal genes, including *MYL9*, *ITGB1*, and *VCL*. In addition, various transcription factors were specifically up-regulated in NLS-actin cells, suggesting a multi-functional role of actin in gene transcription. Down-regulation of adhesion genes was functionally important for cell migration, as the motility of NLS-actin keratinocytes was inhibited both in random cell migration and scratch assays. Conversely, exclusion of actin from the nucleus by siRNA knockdown of Importin 9 enhanced cell migration. Based on these findings, we conclude that the level of actin in the nucleus is an important transcriptional regulator of keratinocyte migration and a novel mechanism for sensing biophysical cues from the adhesive microenvironment.

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Collective endothelial cell migration is regulated by VE-cadherin endocytosis, adhesion, and cytoskeletal linkage

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Angiogenesis, the formation of new vessels from existing vessels, is important during development and during adulthood for wound healing. Dynamic modulation of endothelial cell adhesion is critical for angiogenesis, which occurs by migration and proliferation of endothelial cells. These processes require tight control of adhesion dynamics. However, the mechanisms that regulate junctional plasticity are not fully understood. VE-cadherin is an adhesive protein found in the adherens junctions of endothelial cells. Adhesion occurs through homophillic trans interactions via a conserved tryptophan (W2) in the VE-cadherin extracellular domain. Mutation of this critical amino acid results in the disruption of adhesion. The cadherin cytoplasmic tail binds to members of the catenin family, which stabilize VE-cadherin at the cell surface and increase adhesive strength. Our lab found that mutation of specific amino acids in the VE-cadherin cytoplasmic tail prevents endocytosis of the cadherin and inhibits collective cell migration. We now report that disruption of either adhesion or cytoskeletal linkage relieves the migration suppression caused by the VE-cadherin endocytic mutant. Additionally, mutation of the W2 residue increased VE-cadherin endocytosis relative to wild type cadherin. We observed that lateral dimerization of VE-cadherin prevented endocytosis in the absence of homophilic adhesion. Importantly, dimerized VE-cadherin also inhibited migration in an adhesion dependent manner. Interestingly, we have found that VE-cadherin endocytosis is required for Golgi orientation at the wound edge, a process that is important for directed cell migration. Together, our findings suggest that collective cell migration is cooperatively controlled by VE-cadherin endocytosis, adhesion, and cytoskeletal linkage through the regulation of endothelial cell polarity.

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Plakophilin 1 is Essential for Desmosomal Adhesion and Survival

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Plakophilins 1-3 are components of the desmosomal plaque that link the desmosomal cadherins, desmogleins and desmocollins, to the cytoskeletal linker molecule desmoplakin. Although all three plakophilins are expressed in the epidermis, they display differential expression patterns: Plakophilin 1 is highly expressed throughout all layers of the epidermis with an increase from basal to suprabasal cells. Plakophilin 2 reveals very low expression, whereas plakophilin 3 is expressed at similar levels in all layers. So far it is not known how plakophilins differ functionally and how this affects keratinocyte intercellular adhesion or signaling. A mouse knockout (KO) of plakophilin 3 revealed a very mild phenotype with hair coat abnormalities suggesting that the loss of plakophilin 3 is partially compensated by plakophilin 1. In humans, loss of function mutations in the plakophilin 1 gene lead to skin fragility/epidermal dysplasia accompanied by nail dystrophy, palmoplantar keratoderma, alopecia, and pruritus suggesting defects in epidermal barrier integrity as well as epidermal morphogenesis. In order to elucidate the role of plakophilin 1 in the epidermis in more detail we have generated mice with a targeted deletion of the plakophilin 1 gene. These mice reveal severe skin fragility that appears independent of trauma and die postnatally within one day. Transepidermal water loss is increased indicative of a defective barrier function. Moreover, keratinocytes from these mice reveal reduced desmosomes and decreased intercellular cohesion in an epithelial sheet assay. These data indicate that plakophilin 1 is an essential protein required for stable desmosomal adhesion, epidermal integrity and survival and that its loss is not compensated by either plakophilin 2 or 3.

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The recessive mutation G2375R in human desmoplakin, associated with cardiac, skin and hair abnormalities, inhibits the binding of desmoplakin to intermediate filaments

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Desmoplakin (DP) is an essential component of desmosomes, which are highly specialized cell–cell adhesion protein complexes found in various simple and stratified epithelia, as well as the myocardium. One of the major functions of DP is the anchorage of intermediate filaments (IFs) to desmosomes. In this study, we investigated the effect of the recessive mutation G2375R, found in a patient suffering from arrhythmogenic right ventricular dysplasia, pemphigus-like skin phenotype, and woolly hair, on the interaction of DP with IFs. This mutation is located in the second plakin repeat domain (PRD-B) in the C-terminus of DP. We found in overlay assays and in transfected cells that the mutation G2375R systematically inhibited the binding of full length DP or carboxyl-terminal DP recombinant proteins to various keratins and the muscle-specific IF protein desmin. Yeast three-hybrid and overlay assays also indicated that DP preferentially binds to dimeric/polymeric epidermal keratins 5 and 14 or 1 and 10, compared to monomeric keratins, and the coil 1 domain of dimeric keratins 5/14 and 1/10 is a major binding site for DP. Finally, truncation of the carboxyl-extremity of DP diminished its interaction with all tested IFs. Our findings indicate that the tight interaction of DP with keratins depends on several carboxyl-terminal domains of DP and on the quaternary structure of keratins, mainly of their coil 1. The same rules are also valid for two other plakins, the epithelial bullous pemphigoid antigen 1 (BPAG1e) and plectin, underlying a common binding mechanism between these homologous plakins and IFs.

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Classical cadherin mediated mechanotransduction in the regulation of tight junctions and desmosomes

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Intercellular junctions are essential determinants of tissue architecture and integrity. Previously, we showed that classical cadherins control epidermal integrity and barrier function: Loss of E-cadherin impaired TJs whereas combined loss of both epidermal cadherins, E- and P-, inhibits desmosome assembly. How cadherins control tight junctions and desmosomes is not known. Using whole mount analysis of newborn skin we characterized in vivo junctional organization across layers. This revealed a polarized organization across the epidermis of F-actin, EGFR and vinculin, which was lost In E-cadherin$^{-/-}$ epidermis, coinciding with discontinuous ZO-1 staining. As cadherins serve as mechano-sensors through a-catenin- and vinculin, these results suggest that cadherin mediated mechanotransduction controls TJ assembly and function. In agreement, traction force microscopy revealed a reduction in intercellular tension in E-cadherin$^{-/-}$ keratinocytes and altered cell sorting behavior. Similarly, we found that classical cadherins through a-catenin control intercellular recruitment of the desmosomal proteins desmoplakin (DP) and plakophilin1 (PKP1). Interestingly, intercellular localization of PKP1 or desmomaplakin required different domains of a-catenin, suggesting that their membrane recruitment requires different mechanisms. Impaired formation of TJs and desmosomes was furthermore associated with increased internalization of several TJs and desmosome components. Together, our data indicate that classical cadherins coordinate intercellular junction formation through several mechanisms that couple mechano-sensing, internalization of junctional components and polarized organization of the cytoskeleton and junctions.

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Nuclear expression of IL-33 in skin is positively involved in wound healing in mice

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The skin is the outermost tissue which works as the barrier to external stimuli. Prompt skin wound healing is essential for host defense. The process of wound healing is comprised of complex biochemical and cellular events, and its mechanism still has not been fully elucidated. Our recent study with mouse model revealed high nuclear expression of IL-33 in keratinocytes around the wound site. IL-33 is a member of the IL-1 family of cytokines and is a ligand for ST2L, works as an "alarmin" to stimulate immune reaction following invasive stimuli. In this study, we aimed to reveal the role of IL-33 in skin wound healing. Wild type (WT), soluble ST2 transgenic (sST2 Tg) mice, and IL-33 knockout (IL33KO) mice were wounded by 4mm biopsy punch after removal of hair on the back. From day 1 to day 10 after injury, wound area was measured for comparing the rate of healing. Wound sites were harvested by 6mm biopsy punch. These skin samples were examined histologically and total RNA was extracted from them to analyze the cytokine expression with RT-PCR. IL-33 was time-dependently induced in wound site revealed by RT-PCR and immunostaining. We found no difference in the rate of healing between WT and sST2 Tg, but skin wound healing of IL33KO was delayed compared to WT. The mRNA expression of IL-1β, IL-6, and CXCL1 were markedly enhanced in IL33KO compared to WT, while there was no difference in expression level of these cytokines between WT and sST2 Tg. The delayed wound healing and over expression of IL-6 in IL33KO was improved by intraperitoneal administration of NFκB inhibitor (SN50). Our previous data and others demonstrated that nuclear IL-33 suppressed cytokine production through suppressing activation of NFκB. We speculate that the nuclear IL-33 is positively involved in wound healing, probably by suppressing excessive inflammation through inhibition of NFκB.

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Collagen XII variants in skin repair and fibrosis

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Collagen I is the predominant structural collagen conferring mechanical stability to skin by forming long fibrils that are decorated with numerous proteins including the fibril-associated collagen XII. N-terminal splicing gives rise to collagen XII variants A and B that share high sequence identity at the protein level between human and mouse. High expression of XIIA is detected in embryonic skin, while in adult mice collagen XIIA deposition is restricted to hair follicles. By contrast, collagen XIIB expression persists at basal levels throughout life. Diminished expression or mutation of collagen XII results in impaired connective tissue structure, indicating an important function for collagen XII during the formation or maintenance of a collagenous matrix. Here we aim at identifying distinct roles of the two collagen XII splice variants in situations of elevated collagen deposition and remodeling, i.e. repair and fibrosis of the skin. In contrast to homeostatic skin of mice in which we confirmed the reported expression patterns, large amounts of both collagen XII variants were deposited into the granulation tissue following injury, even prior to deposition of collagen I. Similarly, both splice variants were highly expressed in fibrotic lesions induced by intradermal injection of bleomycin. Mice with forced expression of XIIB in fibroblasts revealed inconspicuous skin morphology at the light microscopic level. Our work demonstrates that collagen XII participates to dermal reconstitution early after injury and indicates that a temporally and spatially orchestrated deposition of distinct collagens might provide a scaffold for proper skin repair. As forced expression of XIIB did not obviously affect skin morphology, XIIA might play a more substantial role. Open questions include which cells release collagen XII early in the healing process, and which is the nature of the superstructure in which collagen XII is found.

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Collagen XVII-laminin-332 interactions modulate keratinocyte motility

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The hemidesmosomal component collagen XVII represents an important epithelial transmembraneous adhesion molecule that is highly expressed at the leading edge of cutaneous wounds and invasive carcinomas. Its collagenous ectodomain is constitutively shed from the cell surface by disintegrin-metalloproteinases (ADAMs). We recently found that shedding of collagen XVII dynamically regulates keratinocyte polarity and directionality during skin regeneration through modulation of laminin-332 deposition and that shedding most likely depends on environmental interactions with laminin-332. Therefore, we wanted to know whether altered laminin-332-collagen XVII interactions disturb directional cell migration. In this study we used keratinocytes from patients with junctional epidermolysis bullosa of late onset, which exclusively produce a collagen XVII mutant with a R1303Q mutation within its extracellular C-terminus. This mutant is normally expressed and showed no defects in membrane targeting and triple helical stability. Since the R1303Q mutation is located within the predicted laminin-332 binding site of collagen XVII we anticipated that it would alter collagen XVII-laminin-332 interactions. Indeed, the JEB pR1303Q collagen XVII keratinocytes were less adhesive and we observed less colocalization of pR1303Q collagen XVII and laminin-332 molecules in immunofluorescence. Furthermore, these cells showed defects in directed cell motility and decreased ectodomain shedding, while the expression rate of integrins α3β1, α6β4 and of laminin-332 was normal. Interestingly, we detected reduced maturation (processing) of laminin γ2. Thus, aberrant collagen XVII-laminin-332 interactions in these cells perturb laminin-332 deposition and maturation, which in turn dynamically coordinates cell polarity and directionality in invasive processes, e.g. during cutaneous wound healing and carcinogenesis.

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Fibroblasts from the elderly produce in vitro fibroplasia slowly, a phenomenon that is enhanced by a fibronectin peptide

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As the elderly population grows, so do the clinical and socioeconomic burdens of non-healing cutaneous wounds, the majority of which occur in persons over 60 years of age. The poor state of the collagenous dermis in the elderly is largely responsible for both the propensity to injury and slowness to heal. To better understand the dysfunctional dermis, we used a cultured adult human dermal fibroblast (AHDF) system that generates in vitro fibroplasia as judged by creation of 3-dimensional layers of fibronectin and collagen matrix (FN/Col) intercalated with fibroblasts. This system generates enough fibroplasia tissue and tension to enable tissue contraction in about one week. In contrast, fibroblasts from the elderly (EHDF) took on average 2 weeks to do the same. FN, a protein synthesized by fibroblasts, is well known to provide a critical link between stromal cells and ECM during tissue formation. Less appreciated is the requirement of FN matrix deposition for collagen matrix deposition and fibroplasia formation. Although EHDF synthesize as much, or more, FN than young AHDF, few studies have addressed the ability of EHDF to deposit FN in the pericellular matrix and generate fibroplasia. We have found in some, but not all, cases that EHDF deposited less FN in the matrix as judged by quantitative immunofluorescence and matrix extraction techniques. In addition, we have found EHDF generated less tension than young AHDF as judged by atomic force microscopy and discrete immunofluorescence speckle correlation/ finite element analysis techniques. Recently we delineated a bioactive FN peptide that supports fibroblast survival and growth. When added to EHDF this 14mer peptide increased EHDF FN matrix deposition, fibroplasia formation, and fibroblast contraction. Our findings strongly suggest that the dermal dysfunction in the elderly results in part by deficient FN/Col matrix deposition and/or inability of flaccid EHDF to contract newly deposited loose extracellular matrix.

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Aging-associated miRNA Impairs Dermal Fibroblast Function Through Inhibition of TGFB1 Gene Expression: A Potential Regulator of Fibrosis

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The function of dermal fibroblasts declines with aging, leading to decreased dermal elasticity, delayed wound healing and wrinkling. We hypothesized that microRNA (miRNA), which consists of small, non-coding RNA molecules involved in RNA silencing and regulation of gene expression, might mediate this age-dependent functional decline. This is important, because such miRNA would be a candidate agent for treatment of fibrosis. To test this idea, we carried out microarray analysis of cultured fibroblasts and skin biopsy samples obtained by laser capture microdissection from subjects of various ages. Microarray analysis of senescent dermal fibroblasts in long-term culture revealed that several miRNAs were increased. Among them, one candidate was also increased in upper-arm skin biopsy samples from aged, but not young, subjects. Transfection of this miRNA into young fibroblasts induced inhibition of TGFB1 gene expression and cellular proliferation, similar to that seen in senescent cells and fibroblasts in aged skin. Binding site prediction suggested that TGFB1 mRNA contains a binding site for this miRNA. Pathway analysis of microarray data showed that this miRNA regulates TGFB1 signaling. Transfection of this miRNA into LL-97 fibroblasts (aberrantly activated fibroblasts from an fibrosis patient) inhibited TGFB1 gene expression and cellular proliferation. Our findings indicate that this senescence-associated miRNA may play a role in age-dependent decline of dermal fibroblast function via inhibition of TGFB1. This finding may provide a basis for development of miRNAs as therapeutic agents for fibrosis.

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Claudin-1 and Occludin are distinctly involved in the pathogenesis of chronic wounds

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Tight junction (TJ) proteins are known to be involved in barrier function, proliferation and differentiation. These processes are essential for normal wound healing and impaired in chronic wounds. Therefore we investigated the TJ proteins Claudin-1 (Cldn-1) and Occludin (Ocln) in tissue samples of acute and chronic human wounds as well as porcine and human ex-vivo wound healing models and after knock-down (KD) in primary keratinocyte cultures. We observed major differences in localization/expression of Cldn-1 and Ocln between normal healing wounds and chronic wounds, with the latter ones often showing an at least partial loss of both proteins at the wound margins while there was an increased expression of Ocln behind the wound margins. In KD experiments we could show that decreased Claudin-1 expression resulted in significantly impaired scratch wound healing, with both, delayed migration and reduced proliferation. ERK, p38-MAPK and AKT pathways are involved. For Ocln, downregulation resulted in increased wound healing in scratch assays which was unexpected, because it is downregulated in chronic wounds. However, after subjecting the cells to mechanical stress – which is normally present in wounds – impaired wound healing was found. This argues for a role of the loss of Ocln in chronic wounds associated with mechanical stress and therefore cell adhesion. In line with this hypothesis, we observed reduced cell-cell and cell-matrix adhesion in Ocln KD cells. Further, also differentiation was altered, while proliferation was unchanged. In summary, we show that there is a difference in expression and localization of Cldn-1 and Ocln between normal wound healing and chronic wounds and both proteins are likely involved in the pathogenesis of chronic wounds. Because this is executed by different effects we conclude that these effects are TJ structure independent but reflect functions of individual TJ proteins in the epidermis.

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TGFβ is released by fibroblasts by regulated secretion involving autophagosomes

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TGFβ is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. While signaling from different TGFβ receptors is well understood, rather sparse information is available on the intracellular trafficking of the ligand and its secretion. Once in the extracellular space, latent TGFβ associates with proteins of the extracellular matrix (ECM) and remains as latent complex until it is activated by integrin-mediated forces opening the latent complex or by proteolysis. Here we deciphered the molecular mechanism of TGFβ secretion by fibroblasts, i.e. the cell type that depends on autocrine TGFβ activity for differentiating into myofibroblasts, which are the essential effector cells in fibrosis depositing the vast amounts of ECM that lead to tissue stiffness. Using gene ablation and silencing in murine and human fibroblasts, we show that release of latent TGFβ1 crucially depends on low RhoA activity, which is controlled by GRAF-1. GRAF-1 localizes to focal adhesions and is maintained in its active conformation by direct interaction with integrin-linked kinase (ILK). Accordingly, fibroblasts with low ILK or GRAF-1 levels display high RhoA and low Rac1 activity and show severely impaired TGFβ secretion. Of note, TGFβ secretion is virtually abrogated upon suppression of autophagosome formation by ablating Atg5 in fibroblasts, indicating a link to regulated secretion by autophagosomes. Indeed, silencing of Rab8, required for this pathway, also impairs TGFβ release. Together, our results demonstrate that in fibroblasts, latent TGFβ is almost exclusively exported via regulated secretion. We have deciphered the molecular mechanism, which is linked to integrins via ILK that is essential for balancing RhoA/Rac1 activity, which in turn regulates autophagosome maturation. Thus, besides their well documented role in TGFβ activation in the extracellular space, integrins are essential for controlling intracellular TGFβ trafficking regulating myofibroblast differentiation and fibrosis.

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Protective effect of botulinum toxin A after cutaneous ischemia-reperfusion injury

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Botulinum toxin A (BTX-A) blocks the release of acetylcholine vesicles into the synaptic space, and has been clinically used for aesthetic indications, neuromuscular disorders and hyperhidrosis. Several studies have demonstrated that BTX-A enhanced the blood flow and improved ischemia in animal models. Our objective was to assess the effects of BTX-A on cutaneous ischemia-reperfusion (I/R) injuries, mimicking decubitus ulcers. Pre-treatment with BTX-A into I/R areas significantly inhibited the formation of decubitus-like ulcer in cutaneous I/R injury mouse model. Furthermore, administration of BTX-A into I/R area just after reperfusion also inhibited the formation of decubitus-like ulcer. The number of $CD31^+$ vessels and αSMA^+ pericytes or myofibroblasts in wounds were significantly increased in the I/R mice treated with BTX-A. The hypoxic area and the number of oxidative stress-associated DNA-damaged cells and apoptotic cells in the I/R sites were reduced by BTX-A administration. In an *in vitro* assay, BTX-A significantly prevented the oxidant-induced intracellular accumulation of reactive oxygen species (ROS) in vascular endothelial cells. Furthermore, the administration of BTX-A completely suppressed the ulcer formation in an intermittent short-time cutaneous I/R injury model, mimicking the pathogenesis of Raynaud's phenomenon. These results suggest that BTX-A might have protective effects against ulcer formation after cutaneous I/R injury by protecting against vascular damage and inhibiting hypoxia-induced cellular damage. BTX-A could be expected to be effective for at least three to six months in humans, therefore, exogenous BTX-A administration has possible long-term preventive and therapeutic potential for patients with cutaneous I/R injuries, including decubitus ulcers and Raynaud's phenomenon-induced digital ulcers.

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p38 MAPK signaling is necessary for desmoglein1 clustering and enhances pathogenic effect, but is not required for blistering in pemphigus foliaceus

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Pemphigus foliaceus (PF) is an autoimmune blistering disease caused by anti-desmoglein1 (Dsg1) IgG. We wanted to determine if p38 mitogen-activated protein kinase (MAPK) signaling and subsequent Dsg1 clustering is necessary for blister formation in PF. Pathogenic (P) and non-pathogenic (NP) anti-Dsg1 monoclonal Abs (mAbs) that were previously isolated as single chain variable fragments by phage display were cloned as IgG mAbs. We compared the effects of a single mAb and a mixture of mAbs on blister formation and the subcellular localization of desmosomal molecules in human skin organ culture. When a P and a NP mAbs were injected together, IgG deposits and Dsg1 localization showed an aberrant granular pattern in the basal and spinous layers and superficial blisters were formed. On the other hand, a single P mAb caused blisters without inducing this clustering of Dsg1. Studies using groups of these P and NP mAbs indicated that Dsg1 clustering required both cross-linking of Dsg1 and trans-interaction blocking by a P mAb. Electron microscopy demonstrated that the desmosome length was shortened by a mixture of a P and a NP mAbs more than a single P mAb. Small molecule inhibition of p38 MAPK blocked Dsg1 clustering but did not block superficial blister formation by a mixture of mAbs. When measured by a dissociation assay, a mixture of a P and a NP mAbs disrupted keratinocytes adhesion more than a single P mAb. This pathogenic effect was only partially suppressed by p38 MAPK inhibitor. These findings indicate that cross-linking of Dsg1 alone without blocking Dsg1 adhesion does not cause blister formation; that p38 MAPK signaling is not necessary for blister formation but enhances it along with Dsg1 clustering. These results suggest that blocking the MAPK signaling pathway alone is unlikely to fully stop blistering in PF patients.

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ArF excimer laser debrides burns without destruction of viable tissue and speeds healing

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Burns and leg ulcers often require debridement to rid the wound of necrotic tissue and possible biofilm formation. This is usually accomplished by surgical or enzyme methods. Complete surgical debridement requires OR time and may lead to removal of viable tissue. Enzyme debridement often causes pain and is usually incomplete. Face, ears, dorsal hands and feet are the most problematic sites for debridement. Therefore we evaluated the ArF excimer laser, with 193nm emission, for burn debridement. Mid- and deep-dermal burns, placed on the flanks of 25kg female swine, were debrided three days after injury with a repurposed Nidek EC-5000 Corneal Surgery System, which was positioned 17cms perpendicularly above the flank at the edge of burns, such that the system's cross-hairs were focused half on the burn and half on normal skin. We used 9 cycles of laser debridement, each cycle lasting 88s, for a total integrated irradiation fluence of 480 Joules/cm^2. Eight mm biopsies of debrided wounds and adjacent normal skin were taken immediately after debridement and 7 days later. In all cases necrotic tissue was removed from burns, while adjacent normal skin showed no evidence of dermal injury. Although the stratified epidermis was removed from the adjacent normal skin, rete ridges remained. Most remarkably, debrided burns showed complete re-epithelialization 7 days later, while non-debrided burns showed little re-epithelialization as judged by histology sections stained with hematoxylin and eosin. These preliminary results strongly suggest that the ArF excimer laser can be used to debride burns with minimal damage to underlying or adjacent normal skin and promote early wound re-epithelialization.

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Non-thermal argon plasma treatment instantly activates β-catenin in the keratinocytes of epidermis leading to the stimulation of skin renewal

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In physics, 'plasma' is the 4^{th} state of the matter, the activated gas which can stimulate various kinds of chemical reactions. Recently, non-thermal plasma (NTP) generating device have been introduced in the field of dermatology. Since NTP has strong anti-pathogenic activity with safeness, NTP was firstly applied on sterilization of skin surface for the better healing of various kinds of skin diseases. However, the effect of NTP on skin regeneration had not been fully explored yet. In this study, the effect of non-thermal argon plasma (NTAP) in the proliferation activity of human keratinocyte was tested. The treatment of NTAP on confluence state keratinocytes helped to escape from G1 cell cycle arrest and increased the cell population at S and G2 phase. In particular, NTAP treatment immediately dispersed the E-cadherin mediated cell-to-cell interaction and by this, E-cadherin associated β-catenin was migrated into the nucleus, leading to the enhanced transcription of its target genes including c-myc and cyclin D1. The NTAP mediated disruption of E-cadherin was fully recovered within 3 hours, representing that the role of NTAP on contact inhibition is reversible event. Moreover, the repeated treatment of NTAP on the mice skin also stimulated epidermal expansion by activating β-catenin of the cells in epidermis. Although the proliferational activity of keratinocytes in basal lamina were enhanced by NTAP, the expression of differentiation markers of keratinocytes such as keratin 10, 14 and involucrin were maintained normally. Fortunately, the symptoms of cellular DNA damage after NTAP treatment were not detected. Taken together, this study elucidated the new property of NTAP, which can be adopted for developing new type of skin-regeneration stimulating devices.

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Effects of fibrinogen, thrombin and ε-aminocaproic acid on the viability of human keratinocytes, fibroblasts and endothelial cells in alginate-based composite matrices

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One of the most desired features of polymeric scaffolds used in skin bioengineering is their ability to support the ingrowth of blood vessels and thus to ensure the effective engraftment of skin equivalent after transplantation. We designed a number of sponge scaffolds using sodium alginate as a basic material with addition of fibrinogen, thrombin and ε-aminocaproic acid as pro-angiogenic microenvironmental components. The scaffolds were crosslinking using glutaraldehyde, Ca^{2+} and Mg^{2+} ions. The porosity of obtained matrixes was 60-70% with pore size between 30-400 μm. The features of the matrices such as degradation rates, changes of mechanical properties and ability to support growth of human keratinocytes (N-TERT), primary fibroblasts and cells spreading from mouse aorta rings (mostly endotheliocytes) in long-term 3D culture have been compared. Cellviability was assessed using fluorescein diacetate labeling. Our results showed that all alginate-fibrinogen matrixes (containing from 15 to 85% of alginate and correspondingly from 85 to 15% of fibrinogen) were effectively colonized by fibroblasts and keratinocytes if their pore size stays between 30 and 150 μm. The introduction of ε-aminocaproic acid actively supports spreading of the mouse aorta ring cells over the matrix volume but blocks the keratinocyte ability to adhere to and fibroblasts ability to migrate into the alginate/fibrinogen scaffolds. The addition of thrombin leads to significant reduction of average pore diameter in alginate/fibrinogen matrixes (less than 50 μm) and supports keratinocyte adhesion and fibroblast migration along with promotion of spreading of mouse aorta ring cells. Thus, in combination with pro-angiogenic components (e.g., fibrinogen and thrombin) alginate-based sponge matrices may represent an efficient (and also cost-effective) alternative to collagen-based scaffolds for skin bioengineering.

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Silk fibroin produced by transgenic silkworms overexpressing the Arg-Gly-Asp motif accelerates cutaneous wound healing in mice

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We investigated the effect of silk fibroin (SF) on wound healing in mice. SF or amorphous SF film (ASFF), prepared from silk produced by the wild-type silkworm *Bombyx mori* (WT-SF, WT-ASFF) or by transgenic worms that overexpress the Arg-Gly-Asp (RGD) sequence (TG-SF, TG-ASFF) was placed on 5-mm diameter full-thickness skin wounds made by biopsy punch on the back of 8 - 12 week-old BALB/c mice. Each wound was covered with WT-ASFF and urethane film (UF), TG-ASFF and UF, or UF alone (control). Wound closure, histological thickness, the area of granulation tissue, and neovascularization were analyzed on days 4, 8, and 12. The effect of SF on cell migration and proliferation was examined *in vitro* by scratch- and MTT assay using human dermal fibroblasts. Wound closure was prompted by TG-ASFF, granulation tissue was thicker and larger in ASFF-treated wounds than the control and neovascularization was promoted significantly by WT-ASFF. Scratch- and MTT assay showed that SF induced the migration and proliferation of human dermal fibroblasts. The effects of TG-ASFF and TG-SF on wound closure, granulation formation, and cell proliferation were more profound than of WT-ASFF and WT-SF. In human dermal fibroblasts WT-SF phosphorylated the MAP kinases ERK1/2, and JNK. We document that SF accelerates cutaneous wound healing and this effect is enhanced with TG-SF.

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Structural and immunological effects of skin cryoablation in a mouse model

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Cryoablation is therapeutically applied for various disorders in several organs, and skin diseases are typical targets as this cryotherapy has been widely used for viral warts, benign tumors, and actinic keratosis. The main mechanisms of cryoablation consist of direct freezing effect on skin constituents, thrombosis formation in microcirculation, and subsequent immunological responses. Among them, however, the immunological mechanism remains unelucidated, and it is an issue how the direct freezing injury induces immunological consequences. We established a mouse cryoablation model with liquid nitrogen applied to the shaved back skin, and used this system to study the immunological excitement. After application of liquid nitrogen, the thermal decrease ratio was -25°C/sec or less and the lowest temperature was less than -100°C, which was sufficient to induce ulceration. Destruction of cornified layer and necrosis of epidermal cells were observed in transmission electron microscopy image, and increased transepidermal water loss and skin permeability were detected by the functional measurements. By flow cytometry, antigen-presenting dendritic cells (DCs), including $PDCA1^+B220^+CD19^-$ plasmacytoid DCs (pDCs) and $CD11c^+$ myeloid DCs, as well as neutrophils and macrophages were increased in subcutaneous tissue. In parallel, the mRNA expressions of interferon α1 which are known as pDC-producing cytokines, was elevated. We also found marked degranulation of mast cells, providing a possibility that released histamine attracts pDCs. Finally, FITC migration assay revealed that pDCs and $CD11c^+$ DCs emigrated from the cryoablated skin to the draining lymph nodes. Our study suggests that cryoablation induces destruction of the barrier/epidermis, accumulation of pDCs and $CD11c^+$ DCs to the skin, and migration of DCs to regional lymph nodes. Viral elements or tumor cell lysates released from damaged keratinocytes may stimulate the DCs, thereby leading to antiviral or antitumor effect.

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Model to study the breakdown of the intermediate filament system under glycation

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Vimentin is a major structural components of the cytoskeleton and one of the intermediate filaments. Vimentin is required for many vital cell functions like cell motility, chemotactic migration and wound healing. However it has been identified as one target for the Advanced Glycation Endproduct (AGE) modification in primary human fibroblast, and carboxymethyllysine-vimentin has been reported to be enhanced in ageing skin. Glycated vimentin is redistributed into perinuclear aggregate and accompanied by a loss of contractile properties of human skin fibroblasts cultured in collagen gel. Here, we demonstrated the accumulation of AGE in glycated fibroblasts, the effect of the glycation on vimentin distribution and we developed a new model to particularly study the consequent loss in mechanical properties. As expected, we observed wide-spread vimentin distribution and fused cellular shape in non-glycated condition, whereas the distribution was concentrated around the perinuclear area evidencing a strong reorganization of cytoskeleton in glycated one. The effect of this disorganization was determined using the analysis of human fibroblasts response to cyclic mechanical stress before and after glycation but also after deglycation. In the model, the majority of fibroblasts reorient themselves, near to the perpendicular stretching direction. But after glycation, fibroblasts were oriented in different directions; no specific direction regarding stretching direction has emerged: glycation diminished fibroblast capacity of reorientation. This effect of glycation can be explained by the fact that glycation induces intracellular and extracellular protein modification namely stiffening. Cytoskeleton's protein like vimentin are modified, organization and adhesion proteins stiffened, reducing then fibroblasts tensing strength and motility. This model has been then used to observe the recovery of all these parameters using a deglycating ingredient that was shown to reverse the structural breakdown of the intermediate filament system due to glycation.

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Restoration of the microfibrillar elastic fibre network in photoaged skin treated with Vitabiotics' Twin Serum, an over the counter anti-ageing formulation

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Photoaged skin is characterised in part by loss of fibrillin-rich microfibrils (FRMs) from the papillary dermis of skin. Topical treatment with all-trans retinoic acid (tRA) improves the clinical appearance of skin, reducing the appearance of fine lines and wrinkles. We have previously shown that tRA also enhances the deposition of FRM which may in part explain the observed clinical improvement. Here we have tested whether a commercially available over the counter anti-ageing product (Perfectil Twin Serum, Vitabiotics Ltd, London) can also induce deposition of FRM utilising a robust occluded patch test assay. Healthy but photoaged volunteers were recruited to the study (n=10; 56-80 years); forearms were treated under occlusion with either Twin Serum (mixed immediately prior to use) or mineral oil vehicle; an untreated occluded area provided a baseline control. Products were applied on d1, d4 and d8 of the assay; tRA was applied to a further site on d8 (positive control). All treated sites were subjected to 3mm punch biopsies on d12 with distribution of FRMs assessed by immunohistochemistry. As with previous studies, tRA induced significant epidermal thickening (n=10; baseline vs tRA, p=0.001) and FRM deposition in the papillary dermis (n =10; mean ± SEM; baseline, 2.60±0.20; tRA, 3.30±0.11; p=0.028). The Twin Serum formula produced deposition of papillary dermal FRMs in 6/10 volunteers highlighting a stratified response to both tRA and to the formula under study (baseline, 2.24±0.19; vehicle, 2.61±0.32; Twin Serum, 2.87±0.24; tRA, 3.50±0.11; p=0.049, repeated measures ANOVA). These data identify the ability of this over-the-counter anti-ageing cosmetic to influence the architecture of photoaged dermis and help induce repair. Since the responses to such cosmetics are person-specific, a stratified or personalised approach to choosing skin care may become an area for further study.